Express Mail Label No.: EL 946407736 US
Date of Deposit: November 2, 2002

Attorney Docket No. 23135-501
(NOV-1)

#17

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Francois Mach		
APPLICATION NUMBER:	**09/664,871**	EXAMINER:	San-ming Hui
FILING DATE:	September 19, 2000	ART UNIT:	1617
FOR:	Statins (HMG-CoA Reductase Inhibitors) as a Novel Type of Immunomodulator, Immunosuppressor and Anti-Inflammatory Agent		

December 2, 2002
Boston, Massachusetts

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. §1.131

I, Francois Mach, hereby declare and state as follows:

1. I am aware that in the Office Action dated August 13, 2002, in the above-identified application ("the Application"), the Examiner has cited Partridge U.S. Patent No. 6,403,637 B1 ("Partridge") under 35 U.S.C. §102(e) as anticipating claims 1-3, 6-7, 11, 13, 15 and 20-32; and under 35 U.S.C. §103(a) as rendering claims 4, 5, 8-10, 16-19 and 35-37 obvious. This declaration is being made to establish reduction to practice of the claimed invention in the Application under 37 C.F.R. 1.131(b) at a date prior to August 9, 1999, the effective filing date of Partridge.

2. I, Francois Mach, invented the invention of independent claims 1, 2, 3, 21, 33, 35, and 37, and hereby declare that in Switzerland, a WTO member country, I reduced to practice the invention claimed in at least these independent claims before August 9, 1999, the effective filing date of Partridge. A copy of the pending claims is attached hereto as Exhibit 1. Note also that a copy of the claims as amended in the accompanying Response to Office Action is attached hereto as Exhibit 2.

3. Reduction to practice of the invention described and claimed in at least independent claims 1, 2, 3, 21, 33, 35, and 37 of the Application is demonstrated by the graphs attached hereto as Exhibit 3. I have described the experiments that produced the data shown in Exhibit 3 below.

4. I analyzed the effect of certain statins on various features of the control of major histocompatibility complex class II (MHC-II) expression and of subsequent lymphocyte activation, to evaluate possible beneficial effects of statins independently of their well-known effect as lipid-lowering agents. Specifically, I studied the effect of statins on the regulation of inducible MHC-II expression by interferon (IFN-γ) in a variety of cell types, including primary cultures of human endothelial cells (ECs). This work is detailed below, and in the data shown in Exhibit 3, and reflects the reduction to practice of the present invention prior to August 9, 1999.

5. **Methods**

Reagents. Human recombinant IFN-γ was obtained from Endogen (Cambridge, Massachusetts). Atorvastatin was obtained from commercial sources.

Cell isolation and culture. Human vascular ECs were isolated from saphenous veins by collagenase treatment (Worthington Biochemicals, Freehold, New Jersey), and cultured in dishes coated with gelatin (Difco, Liverpool, United Kingdom). Cells were maintained in medium 199 (BioWhittaker, Wokingham, United Kingdom) supplemented with 100 U/ml penicillin/streptomycin (BioWhittaker, Wokingham, United Kingdom), 5% fetal calf serum (FCS; Gibco, Basel, Switzerland), 100 μg/ml heparin (Sigma, St. Louis, Missouri), and 50 μg/ml endothelial cell growth factor (Pel-Freez Biological, Rogers, Alaska). Culture media and FCS contained less than 40 pg lipopolysaccharides/ml as determined by chromogenic *Limulus* amoebocyte assay analysis (QLC-1000; BioWhittaker, Wokingham, United Kingdom). ECs were greater than 99% CD31 positive, as characterized by flow cytometry, and were used at passages 2–4 for all experiments.

Flow cytometry. Cells were incubated with FITC-conjugated specific antibody (60 min, 4°C) and analyzed in a Becton Dickinson FACScan flow cytometer (Franklin Lakes, New Jersey). At least 100,000 viable cells were analyzed per condition. Data were analyzed using CELLQUEST software (Becton Dickinson). Cells were treated with nothing (control), atorvastatin (5 μM), and IFN-γ (500 and 1000 U/ml.) Results are plotted in Exhibit 3. The top graph shows MHC-II expression (from left to right) in control cells, atorvastatin-treated

cells (5 μM) and IFN-γ-treated (500 U/ml) cells. The bottom graph shows MHC-II expression (from left to right) in control cells, atorvastatin-treated cells (5 μM) and IFN-γ-treated (1000 U/ml) cells. The graphs show decreased MHC-II expression in atorvastatin-treated cells.

6. These investigations led me to the conclusion that statins act as direct inhibitors of induction of MHC-II expression by IFN-γ and thus as repressors of MHC-II-mediated T-cell activation. This effect of statins is believed to be due to inhibition of the inducible promoter IV of the transactivator CIITA, and observed in cell types including primary human endothelial cells (ECs). The inhibition is specific for inducible MHC-II expression. In repressing induction of MHC-II, and subsequent T-lymphocyte activation, statins therefore provide a new type of immunomodulation. This unexpected effect provides a scientific rationale for using statins as immunosuppressors, not only in organ transplantation but in numerous other pathologies as well.

7. I subsequently confirmed this previously unknown effect of statins in other cell types, including primary human monocyte-macrophages, primary human smooth muscle cells and fibroblasts, as well as in established cell lines such as ThP1, melanomas, and HeLa cells.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Applicant(s): Francois Mach
Appl'n No. 09/664,871

F. Mach

Inventor's Signature

November 25/2002

Date

Full Name of Inventor: Francois Mach
Citizenship: Switzerland
Residence: 24 chemin Petrey, CH-1222 Vesenaz Switzerland
Post Office Address: 24 chemin Petrey, CH-1222 Vesenaz Switzerland

Exhibit 1 – Pending claims

1. A method to achieve MHC-class II mediated immunomodulation in a mammal in need of such treatment, which comprises administering to the mammal at least one statin, or a functionally or structurally equivalent molecule, in an amount effective to modulate MHC class II expression in the mammal.

2. A method to achieve MHC-class II mediated immunosuppression in a mammal in need of such treatment, which comprises administering to the mammal at least one statin, or a functionally or structurally equivalent molecule, in an amount effective to suppress MHC class II expression in the mammal.

3. A method to achieve MHC-class II mediated anti-inflammatory effect in a mammal in need of such treatment, which comprises administering to the mammal at least one statin, or a functionally or structurally equivalent molecule, in an amount effective to suppress MHC class II expression in the mammal.

4. The method of claims 1, 2 or 3, wherein said mammal is a human.

5. The method of claims 1, 2 or 3, wherein said mammal does not suffer from hypercholesterolaemia.

6. The method of claims 1, 2 or 3, wherein said amount is effective to specifically modulate IFN-γ inducible MHC class II expression.

7. The method of claims 1, 2 or 3, wherein said mammal is suffering from a condition which involves IFN-γ inducible CIITA expression.

8. The method of claims 1, 2 or 3, wherein said mammal is suffering from a condition which is an autoimmune disease.

9. The method of claim 8, wherein said autoimmune disease is type I diabetes, multiple sclerosis or rheumatoid arthritis.

10. The method of claims 1, 2 or 3, wherein said mammal is under treatment in preparation of or after an organ or tissue transplantation.

11. The method of claims 1, 2 or 3, wherein said mammal is suffering from a condition which is psoriasis or inflammation.

12. The method of claim 3, wherein said mammal is suffering from a dermatological condition and said statin is used in a topical application.

13. The method of claims 1, 2 or 3, wherein said statin is Compactin, Atorvastatin, Lovastatin, Pravastatin, Fluvastatin, Mevastatin, Cerivastatin, or Simvastatin.

14. The method of claims 1, 2 or 3, wherein said statin, or said functionally or structurally equivalent molecule, has no lipid-lowering effect.

15. The method of claims 1, 2 or 3, wherein the statin, or a functionally or structurally equivalent molecule, is administered in the absence of any other immunosuppressive agents.

16. The method of claims 1, 2 or 3, wherein said amount is comprised between 10 and 80 mg per day.

17. The method of claims 1, 2 or 3, wherein said amount is comprised between 20 and 40 mg per day.

18. The method of claims 1, 2 or 3, wherein said administration comprises intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; or topical, nasal, oral, ocular or otic delivery.

19. The method of claims 1, 2 or 3, wherein said administration is made daily.

20. The method of claim 2 or 3, wherein the immunosuppression or anti-inflammatory effect is the result of repression of T lymphocyte activation.

21. A process for regulating IFN-γ-induced CIITA expression, and CIITA-dependant inter- or intra-cellular events, said process comprising the step of contacting an IFN-γ responsive cell with at least one statin or at least one functionally or structurally equivalent molecule.

22. The process according to claim 21, wherein said contacting is carried out *in vivo* or *in vitro*.

23. The process according to claim 21, wherein said statins are Compactin, Atorvastatin, Lovastatin, Pravastatin, Fluvastatin, Mevastatin, Cerivastatin or Simvastatin.

24. The process according to claim 21, wherein said IFN-γ responsive cell is a cell which has the capacity to become MHC-II positive on induction by IFN-γ.

25. The process according to claim 24, wherein said cell is a primary human endothelial cell, a primary human smooth muscle cell, a fibroblast, a monocyte-macrophage, a cell of the central nervous system, a ThP1 cell, a melanoma cell or a Hela cell.

26. The process according to claim 21, wherein the regulation of IFN-γ-induced CIITA expression is an inhibition of this expression.

27. The process according to claim 21, wherein the regulation of IFN-γ-induced CIITA expression is solely achieved by inhibition of the CIITA inducible promoter IV.

28. The process according to claim 21, wherein said intracellular events comprise induction of MHC-II expression by IFN-γ.

29. The process according to claim 28, wherein the regulation of CIITA expression generates a quantitative regulation of MHC-II expression.

30. The process according to claim 21, wherein said intercellular events comprise MHC-II-mediated T cell activation and proliferation.

31. The process according to claim 21, wherein said regulation can be reversed by addition of L-mevalonate.

32. The process according to claim 21, wherein said regulation of CIITA expression by said inhibitor is dose dependant.

33. A method for identifying molecules that inhibit IFN-γ induced CIITA expression, said inhibition being at least partially reversible by addition of L-mevalonate, comprising the steps of:

-contacting a cell which is IFN-γ responsive with a candidate inhibitory molecule and with IFN-γ;

-detecting the inhibition or absence of MHC class II expression in the presence of the candidate molecule;

-further contacting the cell with L-mevalonate; and

-detecting a total or partial reversal of the inhibitory effect.

34. A method for identifying molecules that inhibit IFN-γ induced CIITA expression, comprising the steps of:

-contacting a cell which is IFN-γ responsive with a statin, or a functional or structural equivalent thereof, and with IFN-γ;

-detecting the inhibition or absence of MHC class II expression in the presence of the statin, or the functional or structural equivalent thereof.

35. A method of treating a patient afflicted with an autoimmune disease, comprising administering to said patient a compound that inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA reductase) in an amount effective to treat said disease.

36. The method of claim 35 wherein said compound has a therapeutically insignificant lipid-lowering effect and suppresses MHC Class II expression.

37. A method of treating a patient suffering from an autoimmune disease or condition comprising:

administering to said patient at least one compound, capable of measurable HMG-CoA reductase inhibition and inhibition of MHC Class II expression in said patient, in an amount effective to treat such autoimmune disease or condition.

38. A method of treating a patient in preparation for or after an organ tissue transplant comprising:

administering to said patient at least one compound capable of measurable HMG-CoA reductase inhibition and inhibition of MHC Class II expression in said patient, in an amount which is effective to prevent tissue rejection.

39. A method of preventing or treating tissue or organ rejection in a patient comprising administering to said patient a compound that inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) in an amount effective to prevent or treat tissue or organ rejection.

Exhibit 2 – Amended claims in Response to Office Action accompanying this petition

1. (amended once) A method to achieve MHC-class II mediated immunomodulation in a mammal with an MHC Class II-mediated inflammatory or autoimmune disorder characterized by IFN-γ inducible Class II transactivator expression, the method comprising administering to said mammal at least one statin selected from the group consisting of compactin, atorvastatin, lovastatin, pravastatin, fluvastatin, mevastatin, cerivastatin, and simvastatin, in an amount effective to modulate MHC class II expression in said mammal.

2. (amended once) A method to achieve MHC-class II mediated immunosuppression in a mammal with an MHC Class II-mediated inflammatory or autoimmune disorder characterized by IFN-γ inducible Class II transactivator expression, the method comprising administering to said mammal at least one statin selected from the group consisting of compactin, atorvastatin, lovastatin, pravastatin, fluvastatin, mevastatin, cerivastatin, and simvastatin, in an amount effective to suppress MHC class II expression in said mammal.

3. (amended once) A method to achieve MHC-class II mediated anti-inflammatory effect in a mammal with an MHC Class II-mediated inflammatory or autoimmune disorder characterized by IFN-γ inducible Class II transactivator expression, the method comprising administering to said mammal at least one statin selected from the group consisting of compactin, atorvastatin, lovastatin, pravastatin, fluvastatin, mevastatin, cerivastatin, and simvastatin, in an amount effective to suppress MHC class II expression in said mammal.

4. The method of claims 1, 2 or 3, wherein said mammal is a human.

5. The method of claims 1, 2 or 3, wherein said mammal does not suffer from hypercholesterolaemia.

6. The method of claims 1, 2 or 3, wherein said amount is effective to specifically modulate IFN-γ inducible MHC class II expression.

10. The method of claims 1, 2 or 3, wherein said mammal is under treatment in preparation of or after an organ or tissue transplantation.

11. The method of claims 1, 2 or 3, wherein said mammal is suffering from a condition which is psoriasis or inflammation.

15. (amended once) The method of claims 1, 2 or 3, wherein said statin is administered in the absence of any other immunosuppressive agents.

16. (amended once) The method of claims 1, 2 or 3, wherein said amount is between about 10 to about 80 mg per day.

17. (amended once) The method of claims 1, 2 or 3, wherein said amount is between about 20 to about 40 mg per day.

18. The method of claims 1, 2 or 3, wherein said administration comprises intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; or topical, nasal, oral, ocular or otic delivery.

19. The method of claims 1, 2 or 3, wherein said administration is made daily.

20. The method of claim 2 or 3, wherein the immunosuppression or anti-inflammatory effect is the result of repression of T lymphocyte activation.

35. (amended once) A method of treating a patient afflicted with an autoimmune disease characterized by IFN-γ inducible Class II transactivator expression, comprising administering to said patient a compound that inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA reductase) in an amount effective to treat said disease.

36. The method of claim 35 wherein said compound has a therapeutically insignificant lipid-lowering effect and suppresses MHC Class II expression.

37. (amended once) A method of treating a patient suffering from an autoimmune disease or condition characterized by IFN-γ inducible Class II transactivator expression comprising:

- administering to said patient at least one compound, capable of measurable HMG-CoA reductase inhibition and inhibition of MHC Class II expression in said patient, in an amount effective to treat such autoimmune disease or condition.

38. A method of treating a patient in preparation for or after an organ tissue transplant comprising:

    administering to said patient at least one compound capable of measurable HMG-CoA reductase inhibition and inhibition of MHC Class II expression in said patient, in an amount which is effective to prevent tissue rejection.

39. A method of preventing or treating tissue or organ rejection in a patient comprising administering to said patient a compound that inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) in an amount effective to prevent or treat tissue or organ rejection.

Exhibit 3

Human Vascular Saphenous Vein Endothelial Cells Stimulated for 48 Hours

IFN 500 U/ml IFN 1000 U/ml Atorv 5 μM